

fusing of the DNA duplex. In order to hybridize SK oligonucleotides (SKO) complementary with the plus strand of the plasmids, they must, of course, represent the sequence of the minus strand, i.e. 5'-CGCTCTAGAACTAGTGGATC-3' (SEQ ID NO: 13). Such an oligonucleotide can be produced by means of automatic oligonucleotide synthesis. These molecules are mixed in an aqueous solution with one of the above-mentioned single-stranded plasmid molecules. Double-stranded regions form at the sites where the SK oligonucleotides (SKO) have found the complementary partner on the single-stranded DNA, i.e. SK oligonucleotide/plasmid hybrids (hereinafter referred to as SKOPH). In order not to impede the binding of the single SKOs to the DNA, a gap of 4 nucleotides is preferably provided as a spacer between the SK oligonucleotide binding sites.

Page 14, line 17, paragraph 3: Please amend as follows:

- a) (SEQ ID NO: 2) Diagram of pBI KS (+). pBI KS (+) was digested with the restriction enzymes Kpn I and BamH I for subsequent cloning. The restriction sites are marked by a finely broken line. The MS fragment therebetween falls out.

Page 14, line 21, paragraph 4: Please amend as follows:

- b) (SEQ ID NOS 2 and 3) Diagram of pBI KS (+) digested with BamH I and Kpn I and the SK-PH I fragment which should result in pBI KS (+) 2x SK by ligation with pBI KS (+). Part of MCS was excised by digest with Kpn I and BamH I (see also a), and the fragment SK-PH I was inserted in return. Using SK-PH I the previously present BamH I restriction site was masked by means of modification of a base pair (bold letters) and a new BamH I restriction site was introduced at the same time. Due to the different restriction sites (Kpn I/BamH I) the fragment can only be cloned in a possible orientation. The restriction site Pvu I served as a control restriction site for the successful incorporation of the insert SK-PH I (no further data shown in this connection).

Page 15, line 6, paragraph 2: Please amend as follows:

- c) (SEQ ID NO: 4) Diagram of pBI KS (+) 2x SK. pBI KS (+) 2x SK was formed by ligation of SK-PH I with the BamH I/Kpn I digested pBI KS (+) (cf. b). The modified BamH I restriction site marked by an asterisk could no longer be excised by BamH I. In order to simplify the following text, the region marked in the illustration (SK primer + non-hybridizing sequence) is marked by a black arrow. This leads to the schematic plan for pBI KS (+) 2x SK as shown under item d).

Page 15, line 20, paragraph 4: Please amend as follows:

Figure 2: simplified diagram of pBI KS (+) 7x SK.

(SEQ ID NOS 10 and 11) The pBI vector is marked by a broken black line; seven SK primer sequences are now contained in its Kpn I/Sac I-oriented MCS. The SK-PH II fragment (dashed arrow on the top and sequence "SK-PH II" emphasized by lines at the bottom) inserted the seventh SK primer and the additional Eag I restriction site in the vector. The important sequences are emphasized in detail. The SK primer sequence is light gray, the rest of MCS and the 4 base spacers are dark gray. The restriction sites are marked in the sequence by a finely broken black line.

Page 16, line 10, paragraph 2: Please amend as follows:

- b) (SEQ ID NO: 12) Presentation of the transitions between individual blocks. The marking of the components can be compared with that in figure 1.a-d. By ligation of the 7x SK block (gray arrow) in the proper orientation, the Not I restriction site which opened the pBI 1x block beforehand was masked by the 5' end of the newly added 7x SK block (bold letters) and could no longer be excised by Not I. The 3' end of the fragment completes the Not I restriction site towards the vector. As a result, it is possible in the next cloning run to again linearize the pBI 2x block with Not I without losing the 14 SK primer. In contrast to the BamH I cleavage site between the individual SK primers in the block (BamH I*), the BamH I restriction site at the 5' end of a 7x block is maintained (BamH I) and can subsequently be used as an orientation control.

Page 16, line 26, paragraph 3: Please amend as follows:

Figure 4: Sequencing result of the plasmid construct containing 27 SK primer elements

(SEQ ID NO: 1) Black bars mark the SK primer sequence regions in the repetitive region sequenced from both sides. Sequences ATCT or GCCG which have a length of 4 base pairs are located between these SK primer sequence regions for reasons of cloning technique.

Page 19, line 21, paragraph 3: Please amend as follows:

In order to accelerate the further cloning steps, the block-wise replication of the SK elements was made by means of polymerase chain reaction (PCR). The plasmid preparation from XL1-Blue was taken as a template DNA for the amplification of the fragment with seven repetitive elements. It was derived directly from the original colony (pBI

7x SK). In a first optimization of the PCR it should be analyzed which primer pair amplified the target fragment having the best quality and quantity. The primers M13, M13 reverse, T3 and T7 (M13: TGTAACGACGGCCAGT (SEQ ID NO: 6); M13 reverse: CAGGAAACAGCTATGACC (SEQ ID NO: 7); T3: AATTAACCCTCACTAAAGGG (SEQ ID NO: 8); T7: TAATACGACTCACTATAGGG (SEQ ID NO: 9)) were tested in various combinations.

Page 26, at the end of the specification, please insert the printed Sequence Listing submitted concurrently herewith.

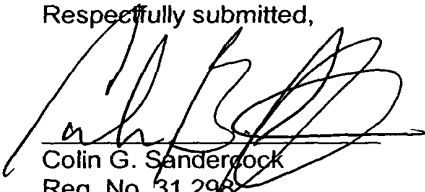
REMARKS

Applicants submit this Amendment to insert the required SEQ ID NOS of the Sequence Listing filed concurrently herewith and to indicate the insertion point for the Sequence Listing.

Applicants respectfully request examination on the merits of this application.

3/1/02
Date

Respectfully submitted,


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